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Interferon-Responsive Regulatory Elements in the Promoter of the Human 2′,5′-Oligo(A) Synthetase Gene

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The interferon (IFN)-activated human 2′,5′-oligo(A) synthetase *E* gene contains 11 RNA starts and lacks TATA and CAAT signals. DNA sequences around the promoter make the expression of the chloramphenicol acetyltransferase gene (CAT) inducible over 20-fold by IFN. A 72-base-pair segment (*E*-IRS) immediately upstream of the RNA starts was defined as being required for IFN-activated expression of the *E*-gene promoter-CAT constructs and acts in a position-independent manner. It also confers IFN-activated enhancement to the herpes simplex virus thymidine kinase promoter. On this promoter, the 5′ part of the *E*-IRS functions as a constitutive enhancer, while the last 16 base pairs of the *E*-IRS is sufficient to give IFN-induced expression. On the *E*-gene promoter, the constitutive enhancer and the IFN-activated sequence are both needed but can be separated. In addition, promoter competition experiments indicate a third regulatory region which helps to repress expression of the *E* gene in uninduced cells.

Cellular responses to interferons (IFNs), such as inhibition of virus and cell growth, require the interaction of these proteins with species-specific membrane receptors (1). This interaction is followed by the induction of a specific set of cellular gene products (reviewed in reference 23), including the 2′,5′-oligo(A) synthetases, which are increased over 100-fold by the different IFNs. We previously reported cloning of a unique human 2′,5′-oligo(A) synthetase gene (*E* gene) which encodes several forms of the enzyme (2, 3). Expression of this *E* gene is involved in the antiviral action of IFNs (5). The mechanism by which IFNs regulate gene activity appears complex. For several IFN-induced genes (10, 14, 18, 25), including the 2′,5′-oligo(A) synthetase gene (14), a rapid transcriptional activation is observed within minutes after exposure of cells to alpha IFN (IFN-α) or IFN-β. However, after 24 h the transcription of these genes is again down-regulated by an active process (18, 19). In this work, we have initiated the study of the type I IFN-dependent activation of the 2′,5′-oligo(A) synthetase *E* gene by identifying sequences which can confer IFN-stimulated expression onto a heterologous promoter and by searching in competition experiments (22) for regulatory DNA elements which keep the gene repressed in uninduced cells. Our results indicate that two transcriptional DNA elements, one of which is IFN activated, and a region binding a putative repressor are involved in the IFN-regulated expression of the 2′,5′-oligo(A) synthetase gene.

MATERIALS AND METHODS

Plasmid constructions. pGEM-CAT-0 was constructed by cloning at the *Hin*dIII site of pGEM-3 (Promega Biotech) a 2.03-kilobase (kb) *Hin*dIII fragment containing the Tn*9* chloramphenicol acetyltransferase (CAT) gene (13) preceded by a 15-base-pair (bp) linker with a *Bgl*II site and followed by the T-antigen intron of simian virus 40 (SV40) (coordinates 4687 to 4080), its poly(A) site (2557 to 2517), and the *Bam*HI-*Hin*dIII segment of pBR322 (Fig. 1). This vector allows easy dideoxynucleotide triphosphate sequencing of deletions, and the multiple unique restriction sites in the polylinker facilitate constructions. For pGEM-CAT-E, the *E*-gene 0.83-kb *Sph*I fragment (3) was cloned in the unique *Sph*I site of pGEM-CAT-0 so as to encode a fusion protein 49 amino acids longer than CAT. For 5′ deletions (5DX series), pGEM-CAT-E was cut with *Kpn*I, digested with BAL 31 (mixed enzyme) for various times, and recut with *Bgl*II. The fragments were sized by agarose gel electrophoresis, electroeluted, and cloned into pGEM-CAT-0, which had been cut with *Sph*I, blunted with mung bean nuclease, and recut with *Bgl*II. For the 3′ deletions (3DX series), pGEM-5D5-CAT was cut with *Bgl*II, treated with BAL 31, and recut with *Kpn*I. The sized fragments were cloned in pGEM-CAT-0 which had been cut with *Sph*I, blunted, and recut with *Kpn*I.

The herpes simplex virus TK gene promoter (−105 to +51) (6) was excised as a *Bam*HI-*Bgl*II fragment from pBL-CAT2 (17) and inserted at the *Bgl*II site between the *E* DNA sequences and CAT of pGEM-CAT-E-3DX plasmids (3DXTK series). For 3D52-5D24 and 3D52-5D29 fusions, pGEM-3D52-CAT was cut with *Bgl*II and dephosphorylated, and the *Bam*HI-*Bgl*II segment of pGEM-5D24 (or 5D29)-CAT was inserted (a *Bam*HI site follows the *Kpn*I site in Fig. 1). About 15 linker residues separate the fused sequences. For cloning in the 3′ end of the CAT-SV40 segment (e.g., 5D29-3D52-3′), the *Bam*HI-*Bgl*II segment of pGEM-3D52 (or 3D51)-CAT was inserted in pGEM-5D29 (or 5D24)-CAT at the *Bam*HI site located after the SV40 poly(A) site (before the *Hin*dIII site; Fig. 1). A segment containing the *Bam*HI site of the polylinker had to be excised before the construction and reinserted afterwards. Plasmids were grown in *Escherichia coli* HB101, and DNA was prepared as recommended by Promega Biotech.

Transfections. HeLa cells (5×10^5 cells) were seeded in 5-cm plates for 24 h, and supercoiled plasmid DNA (10 μg per plate) was transfected (20) by $CaPO_4$ coprecipitation followed after 5 h by glycerol shock. In some experiments, SK cells were similarly used. At 24 h posttransfection, half of the cells were treated with 250 U of pure human recombinant IFN-β_1 per ml (4) and half were mock-treated. Cell extracts were prepared 24 h later for CAT activity assays

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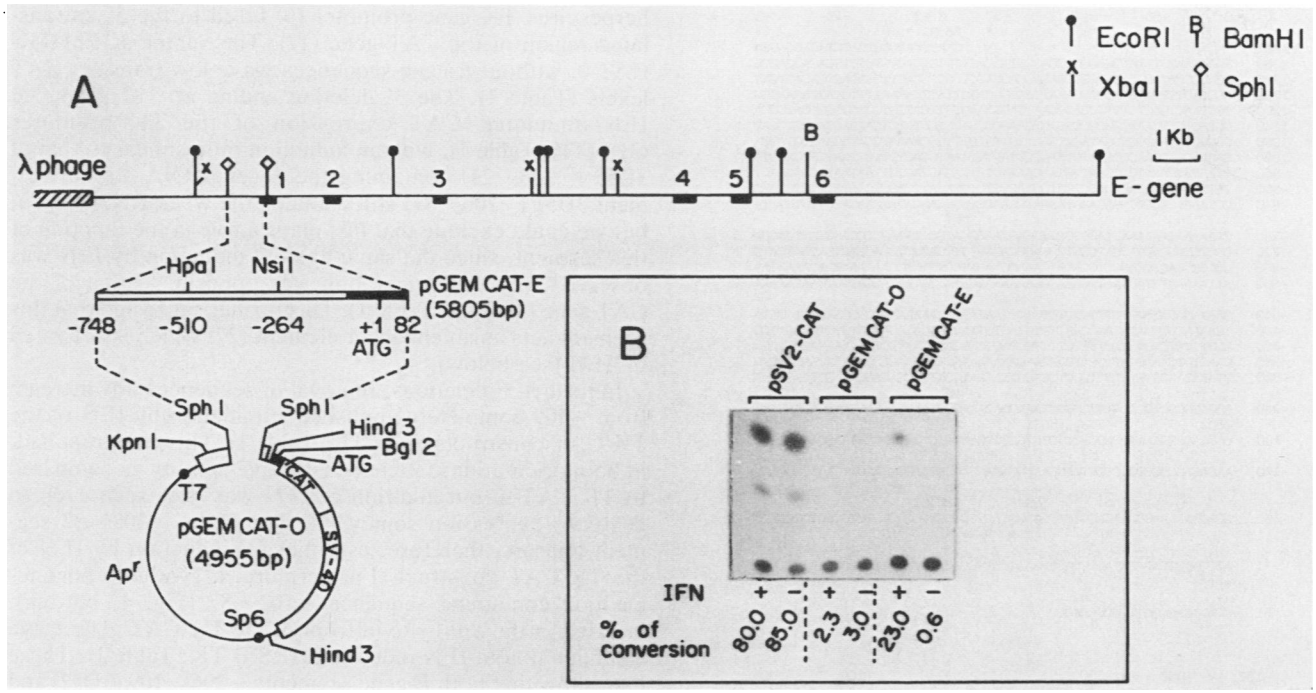

FIG. 1. (A) Map of the human 2',5'-oligo(A) synthetase *E* gene and pGEM-CAT-E plasmid. Exon 1, determined as described in the legend of Fig. 3, contains the 5' end of cDNA 9-21 (1.6 kb), which extends to the end of exon 5, and exon 6 is used by alternative splicing in the 1.8-kb RNA (2, 3). (B) Effect of recombinant human IFN-β_1 on transient expression of CAT activity in HeLa cells.

(13) by incubating 40 μg of protein for 2 h at 37°C with D-threo-dichloroacetyl-[1-^{14}C]chloramphenicol (53 μCi/mmol; Amersham). For competition experiments (22), COS-7 cells were transfected with 5 μg of DNA from pGEM-CAT-E together with 5 μg of pGEM-SV-E containing the SV40 origin of replication followed by the indicated *E* DNA segment in the *Sph*I site of the pGEM-3 polylinker.

RNA 5'-end analyses. DNA fragments were ^{32}P labeled at the *Sph*I site with T4 polynucleotide kinase and hybridized overnight at 42°C in 80% formamide with 6 μg of poly(A)$^+$ RNA from SV80 cells treated for 12 h with 250 U of IFN-β_1 per ml. The hybrids were precipitated with ethanol and dissolved in salt-buffer mixture for reverse transcription as described (20). The primer-extended cDNAs were separated by electrophoresis on 8% polyacrylamide-urea gels. S1 analysis was carried out as described (20).

RESULTS

IFN-inducible 2',5'-oligo(A) synthetase RNA initiation sites and promoter. In the human 2',5'-oligo(A) synthetase *E* gene, the 5' end of full-length cDNAs maps in a 0.83-kb *Sph*I fragment (Fig. 1A), which hybridizes with all four IFN-induced RNA species (2, 3). The sequence of the 5' region of the genomic clone, including this fragment, is shown in Fig. 2. We tested the presence of IFN-regulated promoter sequences by cloning this 0.83-kb *Sph*I fragment in front of a promoterless CAT gene (Fig. 1A) and transfecting HeLa cells with the resulting pGEM-CAT-E DNA. At 24 h after transfection, one culture was treated with recombinant human IFN-β_1, while a parallel culture was left untreated. Cell extracts prepared 48 h posttransfection showed that CAT activity was dependent on treating the cells with IFN, the induction ratio being around 20-fold (Fig. 1B, Table 1). No CAT activity with or without IFN was seen when the 0.83-kb *Sph*I segment was oriented in the opposite direction, or with more 5' genomic fragments in pGEM-CAT-0 (not shown). pSV2-CAT containing the SV40 early gene promoter (13) was as active without or with IFN (Fig. 1B).

To locate IFN-regulated transcriptional start sites in the 0.83-kb *Sph*I segment, we first carried out reverse transcription from a primer sequence, FI (Fig. 3), hybridized to RNA of IFN-treated SV80 cells. This produced multiple cDNAs extending from 100 to 175 bases upstream from the 3' *Sph*I site (Fig. 3, lane 1), suggesting multiple 5' ends. More precise mapping of these 5' ends was obtained by S1 nuclease analysis, which showed that RNA from IFN-treated SV80 cells, but not from untreated cells, protected fragments of multiple sizes ending actually 130 to 178 bp from the 3' *Sph*I site (Fig. 3, lanes 2 and 3 versus lane 4). As shown in the scheme of Fig. 3 (bottom), the 5' ends generated by primer extension were in good agreement with the S1 nuclease analysis, but some were probably due to premature termination. By comparison of the S1 bands with a sequence ladder run in the same gel, the main RNA starts of the IFN-induced transcripts were mapped. Numbering the *E*-gene sequence from the ATG initiator codon (3), the 11 RNA starts detected (a to k in Fig. 2) are clustered between −96 and −48. Site a is weak but corresponds to a reported cDNA for 2',5'-oligo(A) synthetase (26).

In transfected HeLa or SK cells, the IFN-activated transcriptional start sites on the pGEM-CAT-E chimeric construct mapped at the same a to k residues as the authentic *E*-gene transcripts (Fig. 3, lane 6). Complete dependence on IFN treatment was observed (lane 5 versus 6). An additional RNA starting far upstream (z, Fig. 3) was seen also in untreated cells, but represented only a small proportion of the total transcripts. RNA start z follows one of the potential TATA boxes near the *Hpa*I site (Fig. 2), as noticed in a previous analysis (3). In contrast, the IFN-activated multiple

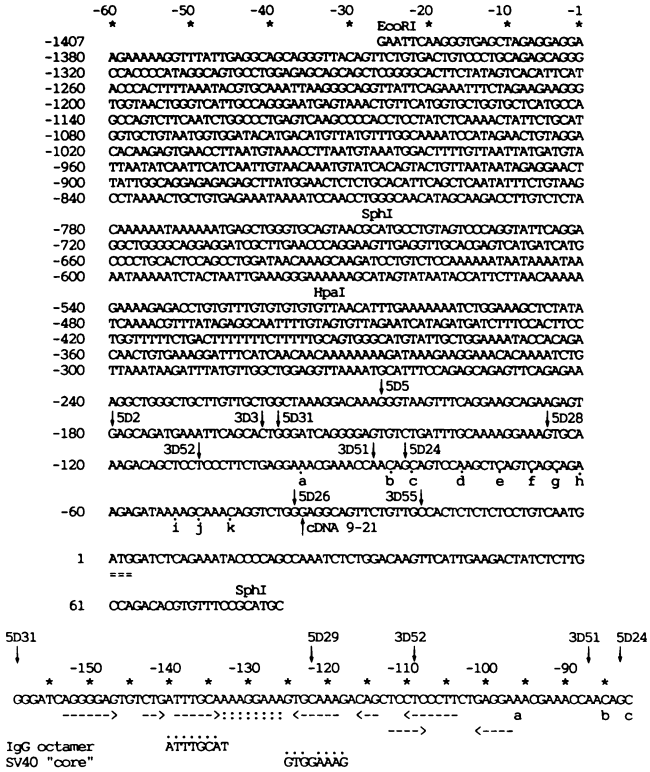

FIG. 2. Nucleotide sequence of the 5′ end of the 2′,5′-oligo(A) synthetase *E*-gene clone containing the *Sph*I 0.83-kb fragment. Sequencing was done according to Maxam and Gilbert (21). Numbering is from the consensus initiator ATG codon (doubly underlined). The 5′ and 3′ ends of deletions are indicated by a down arrow. Letters a to k indicate the main RNA starts (see Fig. 3). Bottom: the *E*-IRS is numbered as described above. Palindromes with dyad symmetry are marked by arrows, and the inverted repeat is indicated by double dots. a, b, and c are RNA starts as described above.

RNA starts a to k are not preceded by TATA boxes (Fig. 2), a finding made also in other eucaryotic promoters with multiple RNA starts (12, 24) and in a mouse IFN-induced gene (25). Transient CAT gene expression from the pGEM-CAT-E DNA, therefore, reflects accurately the activation by IFN of the authentic transcription initiation sites of the E gene.

IFN-regulated sequences in the 2′,5′-oligo(A) synthetase *E* gene (*E*-IRS). A series of 5′ deletions were made on the 0.83-kb *Sph*I fragment (−748/+82) and were tested as described above. In these constructs, the *E*-gene RNA starts and initiator ATG are used to drive the CAT gene. Deletions down to −159 (5D31; Fig. 2) preserved the IFN-dependent CAT expression (Table 1). However, deletion down to −125 abruptly abolished induction in IFN (5D28; Table 1), and further 5′ deletions showed the same phenotype. The constitutive expression without IFN remained low (1 to 4%) in all these 5′ deletions.

To study the effect of 3′ deletions, we first removed the −21/+82 sequences, including the initiator codon and coding sequences. This deletion showed a high IFN-induced expression (3D55; Table 1), but an increase in constitutive expression was noted. We next made deletions removing the main RNA start sites of the *E* gene, which, as expected, produced a marked drop in expression (3D51; Table 1). To test these 3′ deletions, we placed them in front of the entire herpesvirus TK gene promoter (6) fused to the 5′ untranslated region of the CAT gene (17). The vector pGEM-TK-CAT-0, without *E*-gene sequences, gave low transient CAT levels (Table 1). The 3′ deletion ending at −87 produced IFN-stimulated CAT expression of the TK promoter (3D51TK; Table 1), with an induction ratio similar to that of 3D55 (−206/−21) containing the *E*-gene RNA starts. Segment 3D51 (−206/−87) still contains the weak RNA start a, but we could exclude that this plays a role in the function of this segment, since the same fivefold induction by IFN was observed when its orientation was opposite to that of the CAT gene (3DTK/i; Table 1). This further suggested that this segment acts as an enhancer element (27) which is activated by IFN (see below).

In further 3′ deletions, removal of sequences downstream from −109 completely abolished stimulation by IFN of the TK-CAT constructs (3D52TK; Table 1). This construct had, in both orientations, some constitutive activity as compared to TK-CAT-0, but addition of IFN was now seen even to decrease expression somewhat. The 22-bp −108/−87 segment appears, therefore, essential for induction by IFN of the TK-CAT construct. Furthermore, a synthetic oligonucleotide containing sequence −102/−87 (Fig. 4, bottom), inserted in the *Sph*I site in front of the TK-CAT gene, gave a similar sixfold IFN induction (IRS-B TK; Table 1). These data show that both *E*-gene segments −206/−109 (3D52) and −102/−87 (IRS-B) have an effect on the TK promoter: the former (designated segment A) enhances TK-CAT expression but is not stimulated by IFN; the latter (segment B) makes the TK promoter inducible by IFN. However, the results also indicated that with the TK promoter, segment A sequences are dispensable, whereas with the *E*-gene promoter no activity was observed when parts of these sequences were deleted (e.g., 5D28 −125/+82; Table 1). A series of constructs was, therefore, analyzed to confirm the functions of segments A and B on the *E*-gene promoter.

The requirement for segment A for expression of the *E* gene was confirmed by fusing 3D52 (−206/−109) through a 15-bp linker to 5D29 (−122/+82). Neither 3D52 nor 5D29 by itself confers IFN induction, but the recombined 3D52-5D29 fusion showed strong IFN induction (Table 1). The requirement for segment B was confirmed by fusing 3D52 (−206/−109) to 5D24 (−83/+82), providing an internal deletion mutant lacking −108/−84. There was no IFN-induced expression with this 3D52-5D24 fusion (Table 1). To verify the enhancer properties seen with the TK promoter, the entire 3D51 segment (−206/−87), containing both A and B, was placed 3′ of the CAT gene, after the SV40 polyadenylation site at 1.6 kb from the promoter (Fig. 4). For these experiments we used 5D24 (−83/+82), which contains most of the *E*-gene RNA starts in 5′ of the CAT gene and which is not active in IFN-treated cells (Table 1). The resulting construct, 5D24-3D51-3′, showed a sixfold induction by IFN, indicating that both segments A and B are position independent. Furthermore, segments A and B do not have to be contiguous, as shown by 5D29-3D52-3′, which has B 5′ of the promoter and A 3′ after the CAT-SV40 sequences. A clear stimulation by IFN was seen in this construct (Table 1), which contrasts with the lack of response with 5D29 alone. The same is observed with 5D29-3D51-3′ which has B in 5′ and A and B in 3′ (Table 1); the induction is consistently higher with this duplication of segment B.

From these analyses, we conclude that the 72 bp from −159 to −87 contains the IFN-regulated sequences of the 2′,5′-oligo(A) synthetase *E* gene (*E*-IRS; Fig. 2, bottom). The 5′ boundary is defined by 5D31, and the 3′ boundary is

TABLE 1. Effect of *E*-gene 5' flanking sequences on stimulation of CAT gene expression by IFN[a]

Group	Plasmid pGEM-	Limits	CAT activity[b]		Induction ration	*n*
			−IFN	+IFN		
1	CAT-E	(−748/+82)	0.7	17.5	24.0	6
	5A	(−646/+82)	1.4	26.0	18.5	2
	5B	(−305/+82)	1.3	21.7	16.0	2
	5D5	(−206/+82)	1.0	16.2	16.2	5
	5D2	(−180/+82)	2.8	43.1	15.4	2
	5D31	(−159/+82)	1.6	34.0	21.3	5
	5D28	(−125/+82)	1.1	1.1	1.0	3
	5D29	(−122/+82)	0.9	0.9	1.0	2
	5D24	(−83/+82)	0.8	0.85	1.1	2
	5D26	(−37/+82)	0.6	0.8	1.3	2
	CAT-0	No *E* DNA	5.0	2.7	0.54	6
	3D55	(−206/−21)	16.0	64.0	4.0	3
	3D51	(−206/−87)	3.4	8.5	2.5	2
	3D52	(−206/−109)	9.0	2.8	0.3	3
2	3D51TK	(−206/−87)	6.6	36.4	5.5	5
	3D51TK/i	(−87/−206)	10.0	50.0	5.0	2
	3D52TK	(−206/−109)	26.3	19.3	0.73	4
	3D52TK/i	(−109/−206)	35.0	30.0	0.85	2
	B-IRS TK	(−102/−87)	2.1	13.8	6.6	3
	TK-CAT-0	No *E* DNA	1.2	1.0	0.83	3
3	3D52-5D29	(−206/−109)+(−122/+82)	3.0	45.0	15.0	3
	3D52-5D24	(−206/−109)+(−83/+82)	0.3	0.4	1.3	3
	5D24-3D51-3'	(−83/+82)+(−206/−109)−3'	1.2	7.2	6.0	3
	5D29-3D52-3'	(−122/+82)+(−206/−109)−3'	1.1	7.8	7.1	3
	5D29-3D51-3'	(−122/+82)+(−206/−87)−3'	1.5	28.7	19.8	4

[a] HeLa cells were transfected as detailed in Materials and Methods. Ths number of independent experiments used to calculate the expression of each construct is indicated (*n*). The standard deviations for these experiments were 30 to 40% of the mean values. The induction ratios were calculated from the mean. In the experiments of group 2 and 3, an SV40–β-galactosidase chimeric gene was cotransfected, and this activity was used as an internal control. The induction ratio of this control plasmid ranged from 0.5 to 0.8. Constructions are explained by the legend of Fig. 4 and the text.

[b] Results expressed as percent conversion to acetyl chloramphenicol.

defined by 3D51. The *E*-IRS contains two enhancerlike elements, A and B. Both are required for human *E*-gene promoter activity, but element B (defined by the 16-bp IRS-B; Fig. 4, bottom) confers IFN-activated expression on the TK promoter. Element A was never seen to respond alone to IFN induction, but it seems to function as a constitutive enhancer for the TK promoter. The difference between the responses of the TK and *E*-gene promoter constructs could be due to some positive element in TK (such as the CAAT signal) or to an additional control element in the *E*-gene sequences of our CAT constructs. Such a control element could prevent expression in the absence of IFN and mask the enhancer activity of element A. We therefore searched directly for such putative repressor elements.

Derepression in promoter competition experiments. COS-7 cells were transfected with pGEM-CAT-E and with competitor plasmid pGEM-SV-E containing the SV40 origin of replication and various segments of the 0.83-kb *Sph*I fragment of the *E* gene. If repressor DNA elements are present, which would bind proteins exerting a negative effect on *E*-gene expression in the absence of IFN, we would expect to observe derepression of CAT synthesis in the presence of the vast excess of replicating competitor DNA (22). The entire 0.83-kb *Sph*I fragment, used as competitor (Ori-Sph), produced significant derepression in untreated cells as compared to the control competitor vector without *E* DNA (Ori-control) (Table 2). The 3D51 (−206/−87) and 3D52 (−206/−109) segments did not cause significant derepression, indicating that the *E*-IRS segments A and B do not bind factors that repress expression without IFN. On the other hand, 5D29 (−122/+82) caused a marked derepression, reaching 50% of the IFN-induced level of expression. A shorter fragment, 5D26 (−37/+82), was much less active than 5D29, but led to some increase in uninduced expression. The simplest interpretation of these experiments is that sequences binding a putative repressor are present downstream of the *E*-IRS. This could explain the higher constitutive activity of constructs in which the sequences following the RNA starts have been deleted. It could also explain why the constitutive activity of element A is not seen when sequences in 3' of the *E*-gene promoter are present. The competition experiments also indicated that IFN-induced expression of pGEM-CAT-E is reduced by 20% when the plasmid is cotransfected with the Ori-Sph or Ori-5D29 vectors. This decrease could reflect competition for positive factors acting on the *E*-IRS.

DISCUSSION

The promoter region of the human 2',5'-oligo(A) synthetase *E* gene contains about 11 RNA initiation sites, most of which are clustered from −100 to −50 upstream from the initiator ATG codon. No potential TATA and CAAT boxes precede these multiple RNA starts. With 65 bp immediately upstream of the first RNA start, the *E*-gene promoter produces IFN-dependent expression of the chimeric CAT gene in human HeLa and SK cells during transient expression experiments. The IFN treatment induces transcription and accumulation of RNAs initiating on the transfected chimeric DNA at the authentic multiple RNA start sites of the *E* gene. Stimulation of CAT expression was seen at 6 h and was

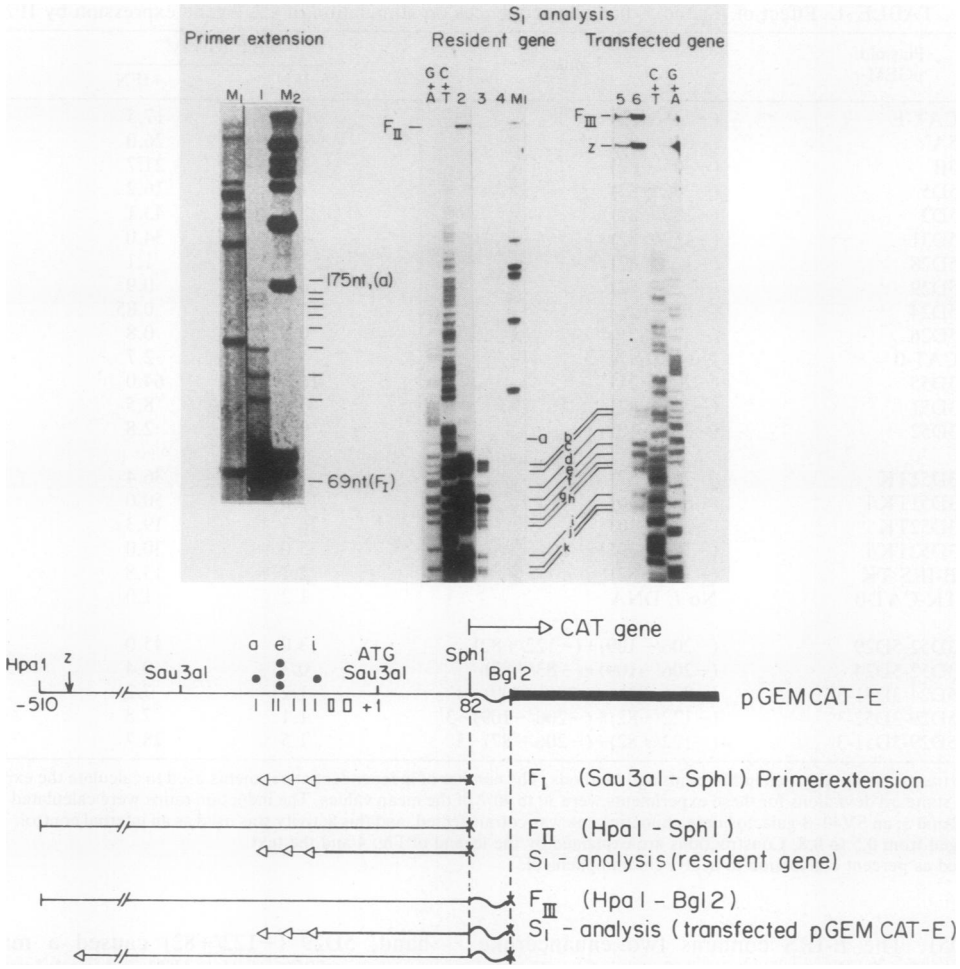

FIG. 3. RNA start sites on the human 2',5'-oligo(A) synthetase *E* gene and transfected chimeric pGEM-CAT-E constructs. The results are schematically shown in the lower panel: black dots indicate intensity deduced from S1 nuclease analysis of RNA starts a, e, and i (Fig. 2). Dashes and rectangles indicate bands from primer extension. Top: Lane 1, primer extension reaction with FI primer; lane M_1, ^{32}P-labeled *Hae*III-cut φX DNA; lane M_2, *Hin*fI-cut pBR322; lanes 2 and 3, S1 nuclease analysis of FII fragment hybridized to 2 μg of poly(A)$^+$ RNA from recombinant human IFN-β_1-treated SV80 cells; lane 4, same but with RNA from untreated cells; lane 5, S1 analysis of FIII fragment (derived from pGEM-CAT-E and 5' labeled at *Bgl*II) hybridized to 20 μg of total RNA from hepatoma SK cells transfected by pGEM-CAT-E but without IFN; lane 6, same as lane 5, but cells were treated from 24 to 36 h posttransfection with 250 U of recombinant human IFN-β_1 per ml. Sequence reactions (21) of FII and FIII fragments are shown (antisense strand). Autoradiography for lanes 3 and 4 was 8 h; that for all others was 48 h.

maximal between 12 and 24 h after IFN-β_1 treatment; it was observed also with IFN-α and IFN-γ (not shown). The 72-bp region −159/−87, designated *E*-IRS (Fig. 2, bottom), could be delimited as required for IFN-induced expression of the *E*-gene promoter. This *E*-IRS, which includes one RNA start but precedes the main cluster of RNA starts, is likely to function in the transcriptional activation of the 2',5'-oligo(A) synthetase gene by IFN detected by in vitro runoff RNA synthesis in nuclei of human cells (14). However, the *E*-IRS is probably not the only regulatory region in the *E* gene, and our results indicate that the very low level of expression in untreated cells may be maintained by an additional DNA element located downstream of the *E*-IRS. The nature of this repression and the question of whether it acts by a transcriptional or posttranscriptional mechanism will be interesting to study.

The *E*-IRS functions as a position- and orientation-independent enhancer element on its own as well as on the heterologous herpes simplex virus TK promoter. It appears to contain two DNA elements, one in the 5' part and one in the 3' part of the *E*-IRS, which are both required for the function of the 2',5'-oligo(A) synthetase gene. The two elements, respectively designated A and B, could be separated and act at distance. When associated with the TK promoter, element A functions by itself as a constitutive enhancer. The first 50 bp of the *E*-IRS, containing element A, never produced an IFN-stimulated response without the presence of the last 22 bp, containing element B. With the TK promoter, element B by itself was also active, and an oligonucleotide representing the last 16 bp of the *E*-IRS conferred IFN-stimulated expression to the TK-CAT constructs. Element B seems also to function as an enhancer which would be activated after IFN treatment of the cell. Element B by itself was not active on the *E*-gene promoter, and neither was element A. The difference between the TK and *E*-gene constructs may result from the presence of CAAT or TATA transcriptional signals in the TK promoter segment used (6). The CAAT box may be able partly to

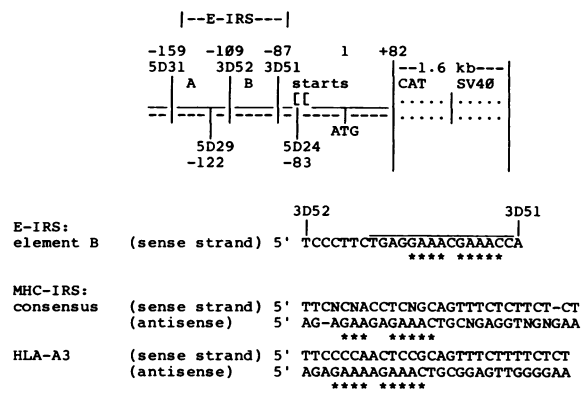

FIG. 4. Top: Schematic representation of the *E*-gene promoter CAT construct. The positions of the *E*-IRS, elements A and B, and RNA starts are shown. Sequences causing derepression in promoter competition experiments are located downstream from the *E*-IRS. Bottom: Sequence comparison between element B of the *E*-IRS and the MHC consensus or HLA-A3 IRS (11). The active *E*-IRS-B oligonucleotide is overlined. Homologous sequences are marked by stars.

replace element A, whose enhancer function would be more stringently required for the *E* gene lacking these transcriptional signals. Other differences, however, are not excluded, as suggested by the additional repressor element in the *E*-gene promoter. No evidence for a repressor function was found in the *E*-IRS, and both elements A and B seem positively regulated.

In search for a common target sequence that would specify IFN-responsive genes, Friedman and Stark (11) have proposed a consensus IRS sequence present in many major histocompatibility complex (MHC) genes and in the metallothionein gene. The 5' flank of the murine MHC class I *H2-K^d* gene, which confers a two- to threefold induction by IFN to chimeric CAT constructs, contains this 28-bp consensus sequence immediately following an enhancer (CRE), and both elements appear necessary for IFN stimulation of the *H2-K^d* promoter (15). The consensus IRS (11) is not present in its entirety in the 2',5'-oligo(A) synthetase gene segment studied here, which shows stronger IFN induction both in CAT constructs and in intact cells than do the class I MHC genes (9). Comparison with the 16-bp IRS-B of the human 2',5'-oligo(A) synthetase gene shows, however, some homology to part of the MHC consensus IRS (Fig. 4, bottom). The decanucleotide GAAACGAAAC is identical in eight positions to a sequence on the antisense strand of the MHC consensus IRS and in nine positions to the corresponding HLA-A3 sequence (Fig. 4, stars). Our data indicate that this oppositely oriented region of the MHC consensus IRS is most likely to be the important IFN-activated target. As in the *H2-K^d* promoter (15), the 5' part of the *E*-IRS can behave as a constitutive enhancer element. This region of the *E*-IRS forms a long palindrome with dyad symmetry centered around an inverted repeat (Fig. 2, bottom) and contains short sequence motifs with seven out of eight base homology to the enhancer octamer element ATTTGCAT of immunoglobulin genes (7) and to the enhancer core sequence of SV40 (27). A G-rich stretch is present as in the H2 CRE enhancer (15). The precise mapping of element A in the 5' part of the *E*-IRS is in progress.

The mechanism by which type I IFNs, after interacting with their cell surface receptors, activate expression of a number of specific genes is yet unknown. It will be important to see whether promoters of other genes strongly activated by these IFNs (16, 19, 25) share common functionally constitutive and IFN-activated sequences with the 2',5'-oligo(A) synthetase gene. Evidence for both positive and negative transcriptional regulation was reported for one such gene (19). Since, in most cells, IFN induces transcription of these genes in the absence of protein synthesis (8, 18), preexisting specific factors may be involved. Evidence for specific protein binding to the isolated −102/−87 segment (element B) of the *E*-IRS was obtained by gel retardation (manuscript in preparation). The significance of the potential 20-bp-long stem-and-loop cruciform structure in segment A of the *E*-IRS for protein binding and the relation of the *E*-IRS to the putative repressor binding site revealed by competition experiments remain to be investigated. Identification of protein binding with the three elements of the 2',5'-oligo(A) synthetase gene promoter reported here will be an important step in elucidating the mechanism of IFN action.

TABLE 2. Effect of promoter competition on expression of pGEM-CAT-E in untreated COS-7 cells[a]

Competitor DNA	Limits	CAT activity[b]	Derepression[c]	*n*
None		3.8	5.2 ± 2.5	8
Ori-control	No *E* DNA	6.5	11.9 ± 5	8
Ori-Sph	− 748/+82	33.1	48.9 ± 15	6
Ori-3D51	−206/−87	4.9	7.2 ± 2.5	2
Ori-3D52	−206/−109	7.2	10.7 ± 5.3	4
Ori-5D29	−122/+82	36.5	55.0 ± 18	8
Ori-5D26	−37/+82	19.3	22.7 ± 15	5

[a] COS-7 cells were cotransfected by pGEM-CAT-E and the indicated competitor vector.

[b] Results are expressed as percent conversion to acetyl chloramphenicol in cells not treated with IFN. Mean was calculated from the number of independent experiments (*n*) indicated in each case. With IFN and no competitor, the mean CAT activity was 62% conversion.

[c] Derepression was calculated as percentage of expression in untreated cells over IFN-induced expression in each experiment.

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